Toxicology 408 (2018) 80–87

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Review

Intimate estrogen receptor-α/ligand relationships signal biological activity

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ARTICLE INFO

Keywords:
Estrogen receptor-α
Estrogen mimics
Xenoestrogens
Conformational change
Ligand interactions

ABSTRACT

How does estrogen receptor-α bind its natural ligands – estrogens? How can other molecules mimic estrogens and elicit different estrogenic responses? The answers lie in a complex and intimate chemical biology between ligands and receptor. This delicate interaction at the ligand binding cleft signals, via conformational change, exposure of a specific new charge topography at a second site (Activation Function-2). This, in turn, attracts a regulatory protein which modulates gene expression and controls biological activity.

1. What do we know about estrogen receptors?

Estrogen receptors (ERs) have functions way beyond traditional ideas of estrogen activity. Not only do they initiate and guide sexual development, they also have key roles in stimulating cell division; for example, early neurological development (Gillies and McArthur, 2010; Heldring et al., 2007b). All of this is achieved by a specialised region of the receptor (binding cleft) which interacts with estrogens to cause conformational changes in the receptor which in turn leads to receptor/ligand complex dimerization followed by occupancy of a DNA region (Estrogen Responsive Element - ERE) which controls gene expression of key genes (Fig. 1) (Shiau et al., 1998).

There are two estrogen receptor isoforms α and β. Structurally they are similar, but their cellular effects are significantly different, perhaps because of their different cell distributions – e.g., breast cancer cells express mainly ERα, whereas gut cells express mainly ERβ (Arai et al., 2000; Okubo et al., 2001). We will focus on ERα in this article because it is the most studied of the receptor isoforms because of its clinical significance (e.g. in breast cancer). ERα is a 17β-estradiol (E2)-activated nuclear receptor (NR3A1 - nuclear receptor subfamily 3, group A, member 1) with remarkably broad ranging effects mediated by occupancy of its ligand binding domain (LBD). ERs have six domains (A–F) including three major functional domains comprising an N-terminal domain which hosts a transcriptional activation function (AF-1), a DNA-binding domain, and a C-terminal LBD (Delfosse et al., 2014; Klinge, 2001).

The LBD comprises two separate, but interacting binding clefts – the ligand binding cleft (LBC) and Activation Function-2 (AF-2) (Brzozowski et al., 1997). Molecular interplay between LBC and AF-2 occupancy determines ERα activity. The LBC binds a ligand (either agonist or antagonist) which initiates a conformational change which exposes AF-2 to allow its interaction with regulatory proteins (Brzozowski et al., 1997). The conformational change initiated by ligand binding initiates ERα dissociation from a heat shock protein (usually Hsp90). Phosphorylation then occurs which aids receptor dimerization. The dimer then moves into the nucleus and binds to DNA via the ERE or via a protein DNA binding intermediate (Murphy et al., 2011). Coregulatory protein recruitment then occurs (Fig. 1).(Shiau et al., 1998). In addition, the coregulatory proteins comprise coactivators (promotors of estrogenicity) and corepressors (suppressors of ER activity). The bound regulatory protein establishes a "triangular relationship" with the ER and the bound ligand (Katzenellenbogen and Katzenellenbogen, 2002) which facilitates fine tuning of the estrogenic response. Furthermore, differential coregulatory protein recruitment contributes to the tissue-specific effects of selective ER modulators (SERMs) (Heldring et al., 2007a).

Occupancy of the LBD can lead to either agonism or antagonism of ERα activity. These two modes of activity are likely to be determined by the manner and strength of binding of ligands to the LBC. For example, a ligand that binds strongly, but does not interact with amino acid residues in a manner that facilitates the receptor conformational change that leads to its interaction with DNA might inhibit E2's agonistic activity - i.e. it blocks the LBC. A good example of an ERα antagonist is 4-hydroxytamoxifen (a cytochrome-P450 catalysed metabolite of the anti-breast cancer drug tamoxifen). It has the molecular attributes necessary to interact with the LBC in a manner akin to E2, but it has a higher relative binding affinity (RBA = 178) than E2 (RBA = 100) (Kuiper et al., 1997) and its phenoxy-*N,N*-dimethylethanamine moiety displaces a common helix that forms the boundary between the LBC and AF-2 (see below) so upsetting AF-2 function. This, of course, explains

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https://doi.org/10.1016/j.tox.2018.07.003
Received 18 February 2018; Received in revised form 5 June 2018; Accepted 5 July 2018
Available online 06 July 2018
0300-483X/

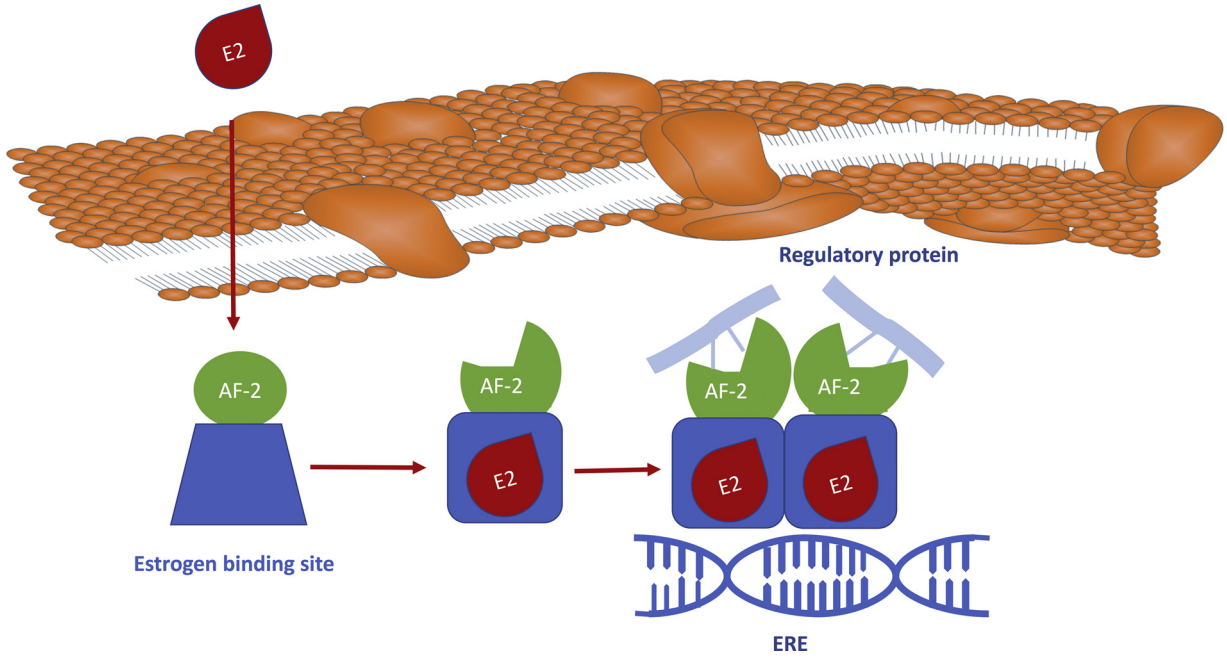

Fig. 1. The mechanism of estrogen bioaction. E2 crosses the cell membrane (orange), interacts with the ERα (blue) LBC which results in a conformational change to the LBC which then results in a knock-on conformation change to AF-2 (green). The resulting conformational change facilitates ERα dimerization and recruitment of regulator protein (pale blue), the entire complex then binds to ERE and initiates gene expression.

why tamoxifen is a very useful treatment for ERα positive (ER +) breast cancer. On the other hand, diethylstilbestrol is a potent ERα agonist; its RBA is 468 (Kuiper et al., 1997) and it has the molecular attributes (see below) to bind to the LBC and initiate the conformational change that leads to ERα activity. This is why diethylstilbestrol was used clinically to minimize the risk of miscarriage until its significant toxic side effects were found.

2. Evolution of ERα: birth of the estrogen mimic

We speculate that ERα evolved in a pristine environment in which it developed an intimate and highly specific relationship with estrogens, particularly E2. The specificity of this relationship was key to female sex hormone function. As time moved on the earth became polluted with chemicals (in 1995 there were 211,934 Chemical Abstract Service (CAS)-registered chemicals, this increased to 88,758,285 by 2006 (Binetti et al., 2008), some of which have molecular analogies with E2. These estrogen analogues can occupy the previously estrogen-specific LBC and thus set the receptor off on its gene regulatory path. These estrogen analogues are termed estrogen mimics (i.e. they mimic the structure of E2 and are either agonists or antagonists) or xenoestrogens (from the Greek ξένος meaning foreign; all agonists) and are now thought to be responsible for human and ecosystem effects like reduced human sperm count, precocious puberty, decreased alligator penis length, and imposex in dog whelks (*Nucella lapillus*)…and all because some man-made pollutants (e.g. the anti-fungal agent used in some cosmetics, methylparaben) and some food plant-derived molecules (e.g. the soy phytoestrogen, genistein) are estrogen lookalikes (Cho et al., 2012; Harris et al., 2005; Lim and Shaw, 2016; Massart and Saggese, 2010; Rider et al., 2010; Sun et al., 2016).

3. What defines a good estrogen mimics?

Estrogen mimics must have molecular characteristics which allow them to fit into, and bind to, the LBC of ERα (Fig. 2). The LBC requires key molecular characteristics of E2 to assure interaction – it's like a 3-dimensional key fitting a 3-dimensional lock; only the key with notches in the right place will open the lock. We know from the structure of E2 that to open the 3-D estrogen lock two hydroxyl groups in the right spatial (i.e. the 17-hydroxyl is in the β position; Fig. 3) orientations, one aromatic and the other aliphatic separated by 9.6 Å of hydrophobicity (Fig. 3) are ideal. Many other molecules have almost the right fit characteristics; e.g., bisphenol A (Fig. 2) has two aromatic hydroxyls 9.3 Å apart and separated by a region of hydrophobicity, genistein has two aromatic hydroxyls 12.1 Å apart and again separated by a region of hydrophobicity. Molecules that can intimately relate to the binding region of the LBC have the greatest estrogenic activity, those capable of less intimacy (e.g. bisphenol A and genistein) (Fig. 2) have lower activities (relative estrogenicities 2.6×10^{-5} and 3.9×10^{-5} respectively where E2 = 1) (Berckmans et al., 2007).

4. ERα has two binding sites

To complicate the process there is a second binding cleft (AF-2) on the receptor which interacts with a regulatory protein, but only when the ligand binding cleft is occupied – we'll explain this later. This is thought to regulate the degree of gene expression that results when the receptor/ligand complex dimer interacts with the ERE on DNA.

Clearly, this is important in an estrogen-mediated growth and development context, but also has profound implications for other estrogen-mediated cellular responses. For example, ER + breast cancer (80% of breast cancers) cells divide in response to E2 and estrogen mimics (Ariazi et al., 2010). Tamoxifen (actually its 4-hydroxy metabolite; Fig. 2) blocks the receptor binding cleft and so inhibits ER + breast cancer cell division, in addition the phenoxy-*N,N*-dimethylethanamine moiety of 4-hdroxytamoxifen bound to the LBC impacts the AF-2 site as outlined above – tamoxifen is the most successful treatment for breast cancer currently available which illustrates the importance of understanding estrogen receptors (Davies et al., 2013).

5. The chemical biology of ligand binding

Highly efficient and specific binding of ligands to the LBC and AF-2 are prerequisites for the desired biological activity of ERα. The specificity of these interactions is mediated by hydrogen bonds between key